PARSEC:
Plant and Rhizobacteria Symbioses to Enhance Crops

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KENNEDY SPACE CENTER
Major: Food Science

NASA Internships and Fellowships Spring Session
04/05/2019

Plant and Rhizobacteria Symbioses to Enhance Crops

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Nomenclature

C	=	Celsius
cfu	=	colony forming units
IMA	=	Inhibitory Mold Agar
ml	=	milliliters
µl	=	microliters
nm	=	nanometers
PGPR	=	Plant Growth Promoting Rhizobacteria
TSA	=	Trypticase Soy Agar

Abstract

Plant Growth Promoting Rhizobacteria (PGPR) are being investigated to determine how to best exploit them to benefit plant production. While they have many potential applications on Earth, researchers at NASA are working to determine if PGPR could be used to improve plant growth in the context of space exploration. Six different bacterial isolates from plant material on the International Space Station were used to inoculate *Brachypodium distachyon* seeds which were then grown and observed. There were no definitively positive growth promoting effects, however this work paves the way for future trials with different variables. If successful relationships between PGPR and food crops can be determined, it would make plant growth for food production much more feasible in beyond Earth environments.

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I. Introduction

Humans have discovered many ways to utilize microorganisms for our own benefit, from the advent of fermented food and drink millennia ago to the development of biofuels for modern applications. Looking to the future, we should continue exploring ways to use our understanding of microbiology as a powerful tool for application to other areas. One potential application of microorganisms is to use them for the improvement of plant growth and crop yield. Plant Growth Promoting Rhizobacteria (PGPR) are a group of bacteria that reside in the rhizosphere and benefit their associated plant in some way. It is a sort of mutually beneficial symbiotic[*] relationship; the bacteria receive a stable supply of carbon in the form of shed root cells which they can break down for energy, while the plant could receive a number of different benefits[1]. Depending on the type of PGPR that has colonized the rhizosphere, the bacteria could provide the plant with fixed nitrogen, plant growth hormones, or pathogen resistance, among other things[1]. This plant-bacteria relationship can be artificially induced by inoculating seeds or live plants with PGPR which may not otherwise have had the chance to colonize the developing rhizosphere of the plant. While PGPR use could be applied to increase crop yields on Earth, it could also be extremely impactful to sustainable space exploration and habitation. One of the major challenges of a long duration space mission is providing the crew with adequate amounts of food. As missions become longer and we venture farther away from our planet, it becomes unrealistic to rely solely on pre-packaged meals for crew members. Limited storage space within spacecraft, limited spacecraft mass at launch, and limited shelf life of pre-packaged meals seem to necessitate some type of supplemental food production during missions. Food crop growth throughout the mission could be an effective way to mitigate some of these issues, but it is not a perfect solution. Growing plants away from Earth is difficult; stressors like microgravity and inconsistent water delivery hinder growth and yield. PGPR should be looked at as one way to minimize the challenges of producing food crops in the hostile environments encountered during space exploration. For example, when zinnia flowers were being grown on the International Space Station, they became infected with a strain of the opportunistic plant pathogen *Fusarium oxysporum*[5]. Many PGPR are capable of out competing pathogens, and some are known to produce antifungals[1]. Additionally, researchers are having a hard time with supplying plants the correct amount of water due to the way water behaves in microgravity[2]. Some PGPR are known to make plants more resistant to drought stress, which could help the situation[6]. Despite the apparent usefulness of PGPR, more research must be performed before their usage can become widespread. Plant-bacteria relationships are complex, and a PGPR that would be beneficial to one type of plant could be detrimental to another[3]. The goal of this research is to discover favorable plant and bacteria combinations, specifically interactions that benefit potential food crops that could be useful during space exploration.

II. *Brachypodium distachyon* and PGPR Trial 1

A. Background

Brachypodium distachyon was the plant selected for the first trial. The advantages of using it include its small size, short life-cycle, hardiness, and relatedness to cereal crops. The six bacteria selected for the first trial were chosen based on their appearance in literature as potential PGPR. Additionally, all bacterial cultures used were originally isolated from plants growing on the International Space Station, to ensure that this experiment would translate well to a microgravity environment. *Burkholderia pyrrocinia* was of particular interest, as it is one of the most common bacterial isolates from International Space Station plant samples. The media selected for plant growth was Murashige-Skoog media with no sucrose added (MS0). The media contained no sucrose or other sugars, which means that the only carbon source available to microbes was shed root cells. The media was also adjusted to a pH of 5.7 as directed on the bottle. The hypothesis was that with shed root cells as the sole carbon source, inoculated bacteria would need to colonize the roots and rhizosphere to survive.

Selected Bacteria

A- *Stenotrophomonas rhizophila*
B- *Burkholderia pyrrocinia*
C- *Methylobacterium rhodinum*
D- *Pantoea agglomerans*
E- *Pseudomonas fulva*
F- *Bacillus amyloliquefaciens*

[*] In some literature, the term symbiotic is reserved for bacteria which physically inhabit plant root nodules. Here it is used to denote a long term relationship between two unrelated organisms.

B. Methods

The selected bacteria were isolated at various times in the past, identified in some way, and stored at -80°C in a glycerol solution. To confirm the identifications were correct, each culture was streaked for isolation on TSA and incubated at 30°C for 48 hours. A single isolated colony from each plate was used for Biolog identification. Gen III biolog plates were used, and the procedure followed was taken from their web site. All isolate identities were confirmed.

The *Brachypodium* seeds used had been stored at 4°C in stable conditions for some amount of time. Before use, the seeds were individually removed from their seed coat/spikelet with tweezers. The seeds were surface sanitized before bacterial inoculation. It is standard procedure to surface sanitize all seeds sent to the International Space Station to minimize Station contamination. There was no existing NASA protocol for sanitizing Brachypodium seeds, so a procedure was adapted from the Hazen lab (University of Massachusetts Amherst) using bleach and Triton X-100 to wash the surface, and rinsing that solution off with sterile DI water.

Cultures of each bacteria were prepared by inoculating TSB with isolated bacterial colonies and incubating overnight on a shaking incubator to use as seed inoculum. Each overnight culture was diluted to 10^8 cfu/ml using a spectrophotometer operating at 540 nm. The target cell density was confirmed by spread plating serial dilutions on TSA plates. After dilution, an equal volume of each inoculum was combined to create a mixed treatment. Seeds were inoculated with 5 ml liquid culture in petri dishes and soaked overnight under a biological safety cabinet. For an unknown reason, the overnight culture of Bacillus amyloliquefaciens did not grow for an additional day, so it was not used during the remainder of this trial.

After soaking overnight, seeds were placed onto MS0 plates with the embryo contacting the agar. Six seeds were placed on each square plate, and each treatment (A, B, C, D, E, Mix, Control) was plated in triplicate. Plates were wrapped in nanopore tape and placed in a reach in growing chamber. The growing chamber was set to 16 hours of light at 22°C and 8 hours of dark at 19°C. The light intensity during light hours was about 100 µmols. Gas composition and pressure was not controlled, and was the same as the ambient room parameters.

Microbiological analysis was performed on the seed inoculum, seeds after soaking, and plant roots after harvest. Seed inoculum micro was performed by spread plating 10-fold serial dilutions of liquid inoculum to confirm target cell density of 10^8 cfu/ml. Soaked seeds micro was performed by vortexing 10 inoculated seeds in sterile DI water, and spread plating 10-fold serial dilutions. This was done to determine the quantity of bacteria picked up by the seeds. Root micro was done by placing all roots from each plate into sterile DI water, breaking up root matter with glass beads, and spread plating 10-fold serial dilutions. Unless otherwise noted, all micro plating was done on TSA and IMA, in duplicate.

C. Results

Qualitatively, there were many visible differences between plants from different bacterial treatments. For example, many of the plants treated with *Burkholderia pyrrocinia* had separate root branches, which is an atypical root structure for *Brachypodium* at that stage of development when compared to controls (fig. 1 and 3). Additionally, 100% of control plants had visible fungal spores on them, while no fungal spores were observed on any PGPR plants with the exception of treatment C (*Methylobacterium rhodinum*). One possible explanation for this is that all or most Brachypodium seeds contained fungal endophytes. After germination, the fungi was out-competed by all inoculated bacteria except for Methylobacterium, which grew too slowly to challenge the fungi. The control seeds had no surface microorganisms present to outcompete the fungi. Another visible deviation from the control plants was the seemingly greater concentration of root hair on certain treated plants, notably the mixed treatment (fig 1 and 2). This could be an effect of the PGPR facilitating root hair production, or it could be the same number of root hairs as the controls have but across a shorter distance, as the control roots were longer.

Figure 1: A control plate showing visible fungal spores

Figure 2: A mixed treatment plate

Figure 3: A *Burkholderia pyrrocinia* plate

Qualitatively, the data shows that the only significant differences in shoot fresh weight was negative (fig 4). Similarly, the only significant differences in root length were negative (Fig 5).

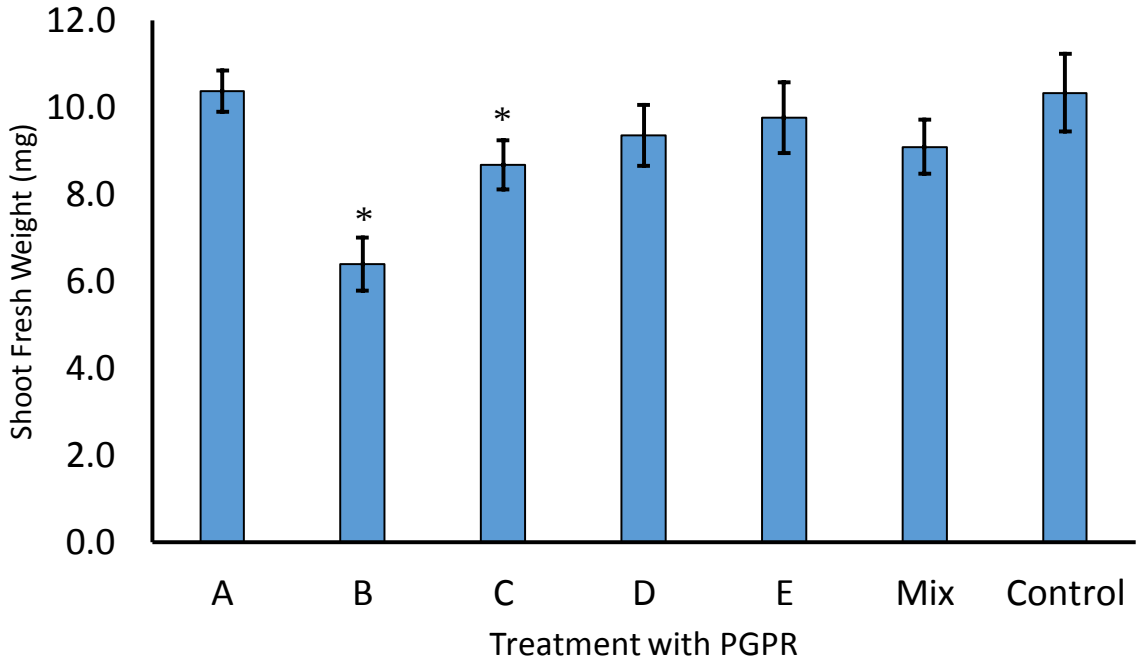

Figure 4: Graph comparing shoot fresh weights across treatments

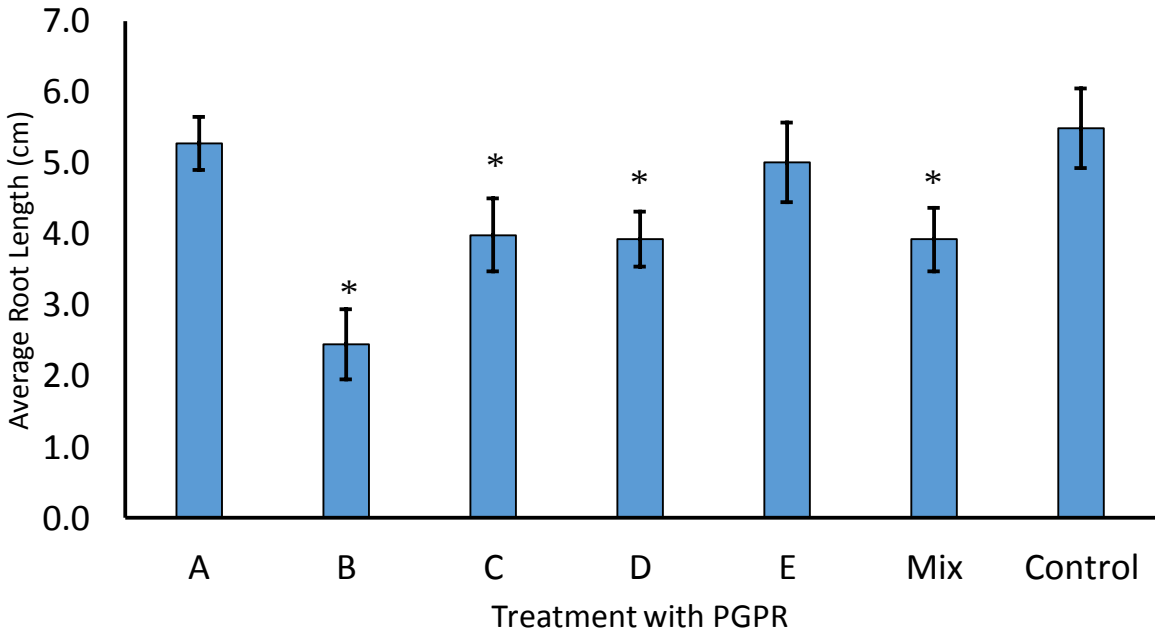

Figure 5: Graph comparing shoot lengths across treatments

III. Conclusion

Looking at the results of this trial, we determined that our treatment is not optimized in some way. There were no increases in shoot fresh weight or root length after treatment with PGPR, and in fact treatment seemed to detriment plant health in most cases. One possible explanation is that the bacterial concentrations the seeds were exposed to was too high. Another thought was that these PGPR may work more efficiently with lettuce, which many of them were isolated from. In the immediate future, lettuce trials and concentration trials will be performed. At this time, additional research into PGPR seems beneficial to future space exploration.

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